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=> d his

     (FILE 'HOME' ENTERED AT 17:36:20 ON 27 MAY 2004)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS,
     DDFB, DDFU, DGENE, DRUGB, DRUGMONOG2, ...' ENTERED AT 17:37:43 ON 27 MAY
     2004
                SEA 2,5-DIKETO-L-GLUCONIC ACID REDUCTASE
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               1    FILE BIOSIS
               2    FILE IFIPAT
               2    FILE USPATFULL
               1    FILE WPIDS
               1    FILE WPINDEX
L1              QUE 2,5-DIKETO-L-GLUCONIC ACID REDUCTASE
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     FILE 'IFIPAT, USPATFULL, BIOSIS, WPIDS' ENTERED AT 17:42:36 ON 27 MAY 2004
L2             5 S L1 AND ASCORBIC
L3             3 DUP REM L2 (2 DUPLICATES REMOVED)
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=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 3 DUP REM L2 (2 DUPLICATES REMOVED)

=> d l3 ibib ab 1-3

L3 ANSWER 1 OF 3 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 1
AN 10459023 IFIPAT;IFIUDB;IFICDB
TITLE: NOVEL **2,5-DIKETO-L-GLUCONIC ACID REDUCTASES** AND METHODS OF USE
INVENTOR(S): Donnelly; Mark, Warrensville, IL, US
Eschenfeldt; William H., St. Charles, IL, US
Trent; Jonathan, La Silva Beach, CA, US
PATENT ASSIGNEE(S): Unassigned
AGENT: Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA, 94034-1013, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003203449	A1	20031030
APPLICATION INFORMATION:	US 2003-418401		20030417

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 2000-684385	20001004	6576452
FAMILY INFORMATION:	US 2003203449	20031030	
	US 6576452		

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 24 8 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1 shows the alignment of the nucleotide sequences of the six environmental DNA PCR products. The entire sequence of clone pI-14 is shown. Identical bases in the remaining sequences are indicated by dots (.). Gaps introduced into the alignment are indicated as dashes (-). The solid bars indicate the locations of the two degenerate PCR primers.
FIG. 2 shows the nucleotide sequences of the full-length clones for pI-14 (FIG. 2A) and pI-28 (FIG. 2B). The coding region for the putative reductase genes are indicated in capitol letters with the deduced amino acid sequence shown immediately underneath in single letter code. Locations of the degenerate and clone-specific primers are indicated by arrows. The putative partial open reading frames upstream and downstream from the reductase gene are indicated by solid bars.
FIG. 3 shows the alignment of the deduced amino acid sequences of clones pI-14 and pI-28. The entire sequence of pI-14 is shown. Identical bases in clone pI-28 are indicated by dots (.).
FIG. 4 depicts a recombinant process for the conversion of glucose to ***ascorbic*** acid.
FIG. 5 depicts mass spectra of 2-keto-L-gulonic acid reaction product and 2-keto-L-gulonic acid standard. FIG. 5A shows the mass spectrum of the 2-keto-L-gulonic acid reaction product. FIG. 5B shows the mass spectrum of the 2-keto-L-gulonic acid standard.
FIG. 6 depicts the dependence of the rate of reaction on pH.
FIG. 7 depicts the NADH-dependent 2,5-diketo-D-gluconic acid activity of environmentally isolated 2,5-diketo-D-gluconic acid reductases. FIG. 7A shows the NADH dependent activity and FIG. 7B illustrates enhancement of NADH-dependent activity by inclusion of inorganic phosphate.
FIG. 8 depicts the thermal stability of 2,5-diketo-D-gluconic acid reductase environmental form d (DKGRd).

AB Described herein are novel nucleic acids, proteins and methods that can be used to provide new catalysts with desirable traits for industrial processes. In particular, novel reductases isolated from the environment using PCR methods are described.

L3 ANSWER 2 OF 3 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 2

AN 03891485 IFIPAT;IFIUDB;IFICDB

TITLE: **2,5-DIKETO-L-GLUCONIC ACID REDUCTASES** AND METHODS OF USE

INVENTOR(S): Donnelly; Mark, Warrensville, IL
Eschenfeldt; William H., St. Charles, IL
Trent; Jonathan, La Silva Beach, CA

PATENT ASSIGNEE(S): Genencor International, Inc., Palo Alto, CA, US

PRIMARY EXAMINER: Achutamurthy, Ponnathapu

ASSISTANT EXAMINER: Pak, Yong

AGENT: Ito Richard T.

	NUMBER	PK	DATE
PATENT INFORMATION:	US 6576452	B1	20030610
APPLICATION INFORMATION:	US 2000-684385		20001004
FAMILY INFORMATION:	US 6576452		20030610

DOCUMENT TYPE: Utility
Granted Patent - Utility, no Pre-Grant Publication

FILE SEGMENT: CHEMICAL
GRANTED

GOVERNMENT INTEREST:

This invention was made with United States Government support under Award No. 70 NANB 5H1138 awarded by the United States Department Of Commerce. The Government has certain rights in this invention.

NUMBER OF CLAIMS: 11

GRAPHICS INFORMATION: 8 Drawing Sheet(s), 12 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 shows the alignment of the nucleotide sequences (SEQ ID NO:1-6) of the six environmental DNA PCR products. The entire sequence of clone pI-14 is shown. Identical bases in the remaining sequences are indicated by dots (.). Gaps introduced into the alignment are indicated as dashes (-). The solid bars indicate the locations of the two degenerate PCR primers.

FIG. 2 shows the nucleotide sequences of the full-length clones for pI-14 (FIG. 2A (SEQ ID NO:7)) and pI-28 (FIG. 2B (SEQ ID NO:9)). The coding region for the putative reductase genes are indicated in capitol letters with the deduced amino acid sequence (SEQ ID NO:8 and 10 respectively) shown immediately underneath in single letter code. Locations of the degenerate and clone-specific primers are indicated by arrows. The putative partial open reading frames upstream and downstream from the reductase gene are indicated by solid bars.

FIG. 3 shows the alignment of the deduced amino acid sequences of clones pI-14 (SEQ ID NO:8) and pI-28 (SEQ ID NO:10). The entire sequence of pI-14 is shown. Identical bases in clone pI28 are indicated by dots (.).

FIG. 4 depicts a recombinant process for the conversion of glucose to ***ascorbic*** acid.

FIG. 5 depicts mass spectra of 2-keto-L-gulonic acid reaction product and 2-keto-L-gulonic acid standard. FIG. 5A shows the mass spectrum of the 2-keto-L-gulonic acid reaction product. FIG. 5B shows the mass spectrum of the 2-keto-L-gulonic acid standard.

FIG. 6 depicts the dependence of the rate of reaction on pH.

FIG. 7 depicts the NADH-dependent 2,5-diketo-D-gluconic acid activity of environmentally isolated 2,5-diketo-D-gluconic acid reductases. FIG. 7A shows the NADH dependent activity and FIG. 7B illustrates enhancement of NADH-dependent activity by inclusion of inorganic phosphate.

FIG. 8 depicts the thermal stability of 2,5-diketo-D-gluconic acid reductase environmental form d (DKGRd).

AB Described herein are novel nucleic acids, proteins and methods that can be used to provide new catalysts with desirable traits for industrial processes. In particular, novel reductases isolated from the environment using PCR methods are described.

L3 ANSWER 3 OF 3 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-463231 [49] WPIDS
DOC. NO. CPI: C2002-131645
TITLE: Diketo-D-gluconic acid reductases, isolated from the environment using polymerase chain reaction methods, useful to provide new catalysts with desirable traits for industrial processes.
DERWENT CLASS: B03 B04 D16 E13
INVENTOR(S): DONNELLY, M; ESCHENFELDT, W H; TRENT, J
PATENT ASSIGNEE(S): (GEMV) GENENCOR INT INC; (DONN-I) DONNELLY M; (ESCH-I) ESCHENFELDT W H; (TREN-I) TRENT J
COUNTRY COUNT: 98
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002029019	A2	20020411	(200249)*	EN	58
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2002011843	A	20020415	(200254)		
US 6576452	B1	20030610	(200340)		
EP 1322753	A2	20030702	(200344)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					
US 2003203449	A1	20031030	(200372)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002029019	A2	WO 2001-US42445	20011002
AU 2002011843	A	AU 2002-11843	20011002
US 6576452	B1	US 2000-684385	20001004
EP 1322753	A2	EP 2001-979928	20011002
		WO 2001-US42445	20011002
US 2003203449	A1 Div ex	US 2000-684385	20001004
		US 2003-418401	20030417

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2002011843	A	Based on	WO 2002029019
EP 1322753	A2	Based on	WO 2002029019
US 2003203449	A1	Div ex	US 6576452

PRIORITY APPLN. INFO: US 2000-684385 20001004; US 2003-418401 20030417
AB WO 200229019 A UPAB: 20020815
NOVELTY - An isolated polypeptide (I) comprising 2,5-diketo-D-gluconic acid reductase (DKGR) activity and comprising an amino acid sequence with at least approx. 60% identity to clone pI-14 and clone pI-28 with fully defined 275 (S1) and 265 amino acid sequences respectively, given in the specification, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the

following:

(1) an isolated nucleic acid molecule (II) comprising a nucleic acid sequence which encodes (I);

(2) an isolated nucleic acid molecule (III) comprising a sequence with at least approx. 60% sequence identity to a sequence (S2) of 368, 359, 365 or 398 nucleotides fully defined in the specification;

(3) an expression vector (IV) comprising (II) or (III);

(4) a host cell (V) comprising (IV); and

(5) identifying **2,5-diketo-L-gluconic acid reductase**, by isolating nucleic acid molecules with homology to **2,5-diketo-L-gluconic acid reductase** from uncultured microorganisms, and screening the molecules for DKGR activity, where the molecules with DKGR activity are identified as **2,5-diketo-L-gluconic acid reductase.**

USE - (V), in particular Pantoea sp., is useful for converting glucose to **ascorbic** acid, by culturing the host cell under conditions suitable for the expression of DKGR (claimed). DKGR nucleic acids and proteins are useful to make enzymes useful in industrial processes to convert glucose to vitamin C in a single organism. DKGR proteins or their fragments and derivatives are useful as immunogens to produce antibodies useful in screening for similar enzymes from other organisms and samples. These antibodies are employed to screen gene libraries to identify DKG reductases or cross reactive activities. (II) is useful to obtain additional coding and non-coding regions and to make a variety of expression vectors to express DKGR proteins which can then be used to convert DKGR to 2-keto-L-gluconic acid. DKGR nucleic acids may be sequenced and subjected to site specific mutagenesis to develop modified DKGR with desired properties that are absent or less pronounced in the wild-type proteins, such as stability to heat, solvent tolerance, NADH dependent activity and different optimum pH.

ADVANTAGE - (I) has improved catalytic efficiency, improved thermal stability, increased solvent tolerance and altered pH optimum (claimed).

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